Analysis of the serum levels of heat shock protein 70 (HSP70) after inhalation of a steam-gas mixture containing hydrogen gas

Nobunao IKEWAKI, Tohru SONODA*, Hiromichi YAMAO**

Abstract

In this study, we examined the effects of inhaling a steam-gas mixture containing hydrogen gas on the serum levels of heat shock protein 70 (HSP70), an inflammatory biomarker, in 19 healthy adult volunteers. The steam-gas mixture containing hydrogen gas, called "ENEX," was generated through the decomposition of superheated steam using an instrument manufactured by Suisonia Co. (Kitakyushu, Japan). Serum HSP70 levels were measured using an enzyme immunoassay after the subjects inhaled "ENEX" via a nasal cannula for 60 min. Serum HSP70 levels significantly decreased ($P < 0.05$) in both male and female volunteers after inhaling "ENEX" . However, serum levels of total IgM did not change after inhaling "ENEX" . These findings indicate that inhaling "ENEX" effectively and rapidly decreased the serum levels of the inflammatory biomarker, HSP70, and may therefore exert therapeutic effects against various inflammation-related diseases.

Key words : ENEX, heat shock protein 70 (HSP70), hydrogen gas, inflammation, total IgM

Introduction

Reactive oxygen species (free radicals) have been implicated in the development of many diseases. Recently, it was reported that removal of reactive oxygen species by molecular hydrogen (H_2) may be one of the most effective methods to prevent and treat various diseases. In fact, many studies have demonstrated that inhalation/ingestion of H_2 improves the clinical and pathological manifestations of numerous human diseases [1]. H_2 could be administered as antioxidant therapy, either orally as H_2 water, or by inhalation as 2% - 4% H_2 gas [2]; in particular, the antioxidant efficacy of H_2 gas inhalation has been investigated in several medical fields [3, 4]. In addition, since oxidative stress induces both acute or chronic inflammatory responses, H_2 gas ingestion/inhalation could be an effective therapy, owing to its antioxidant effect, for various diseases related to inflammation. However, the effects of H_2 gas inhalation, in particular, the kinetic changes of several inflammatory factors in the living body are still very poorly understood.

Recently, a steam-gas mixture containing H_2 gas generated by decomposition of superheated steam was developed by Suisonia Co. and designated as "ENEX" at our laboratory. This steam-gas mixture containing H_2 gas is known to be stable and safe and is widely used in many medical institutions.

The aim of the present study was to analyze the effect of "ENEX" inhalation on the serum levels of the inflammatory biomarker, heat shock protein 70 (HSP70), which is induced by oxygen species in the living body. In addition, we also discuss the possibility of using the beneficial effects of "ENEX" to prevent or treat various diseases associated with inflammation.

Department of Medical Life Science, Kyushu University of Health and Welfare School of Medical Life Science, Institute of Immunology, Junsei Educational Institution, 1714-1 Yoshino-cho, Nobeoka-city, Miyazaki, 882-8508 Japan
*Department of Occupational Therapy, Kyushu University of Health and Welfare School of Health Science, Institute of Immunology, Junsei Educational Institution, 1714-1 Yoshino-cho, Nobeoka-city, Miyazaki 882-8508, Japan
**Inoue Hospital of Obstetrics and Gynecology, 1-990-1 Hirabaru-cho, Nobeoka-city, Miyazaki 882-0866, Japan

Materials and Methods

Reagent

The enzyme immunoassay (EIA) kit for measurement of HSP70 was purchased from Cosmo Bio Co. (Tokyo, Japan).

Machine used for generation of the steam-gas mixture containing H_2 gas

A machine (FRJ-003) developed by Suisonia Co. (Kitakyushu, Japan) that decomposes superheated steam to produce a steam-gas mixture containing H_2 gas was used in this study. When superheated steam produced by heating water is further superheated, it decomposes into hydrogen (H_2) and oxygen (O_2), at a decomposition ratio of 67% H_2 and 33% O_2 (water: 100%). Since air is also present inside the machine, the concentration of H_2 gas produced within the machine is approximately 2.4%. Because the H_2 produced is delivered by introducing air, the inhaled H_2 concentration is approximately 0.1% to 0.3%. O_2 is also produced by the decomposition, but the H_2 is isolated by using a cartridge to adsorb the O_2. This steam-gas mixture containing H_2 gas is designated as "ENEX" at our laboratory.

Ethics statement

The study protocol was approved by the institutional review board (IRB) of Kyushu University of Health and Welfare (IRB number 20-019). Informed consent was obtained from each of the healthy volunteers prior to their participation in this study.

Volunteers and serum collection

Nineteen healthy volunteers (13 male and 6 female; age 38.2 ± 12.1 yr and age 44.8 ± 6.6 yr, respectively) were selected for this study at Inoue hospital. While the subjects were seated on a chair, "ENEX" was administered by inhalation via a nasal cannula for 60 min. Blood samples were obtained from all the volunteers both before and after the inhalation. The blood samples were centrifuged at 2,500 rpm for 10 min, and the sera were collected and stored at -80℃ until the assays.

Measurement of HSP70 in the serum

The HSP70 levels in the serum samples were measured using the HSP70 detection EIA kit. Each of the measurements was performed in triplicate.

Measurement of serum total IgM

Measurement of serum total IgM in the samples was outsourced to a Special Reference Laboratory (SRL). Each measurements was performed in triplicate.

Statistical analysis

Data were analyzed statistically by Wilcoxon' s *t*-test. Differences at $P < 0.05$ were considered as being statistically significant.

Results and Discussion

We examined the effect of "ENEX" inhalation on the kinetic changes of HSP70 in the serum. The 19 normal healthy volunteers (13 males and 6 females) inhaled "ENEX" for 60 min, followed by measurement of the serum HSP70 levels by EIA. As shown in Figure 1, the serum HSP70 levels in the male volunteers decreased significantly ($P < 0.05$) after the "ENEX" inhalation as compared to the levels recorded before the "ENEX" inhalation (panel A). Furthermore, the serum HSP70 levels also decreased significantly in the female volunteers ($P < 0.05$) (panel B). These results strongly suggest the absence of any significant gender difference in the decrease of the serum HSP70 levels after "ENEX" inhalation. On the other hand, no significant changes in the serum HSP70 levels were observed in the absence of "ENEX" inhalation (data not shown). Furthermore, as shown in Figure 2, no significant changes in the serum total IgM levels were observed after "ENEX" inhalation either in the male

volunteers (panel A) or the female volunteers (panel B) under the same conditions.

HSP70 is a specific protein that is expressed when cells are briefly exposed to high temperatures exceeding their normal growth temperature, and exposure to lethal temperatures induces apoptosis. The synthesis and production of HSP70 is a universal phenomenon, occurring in all plant and animal species, including humans. HSPs can also be induced by exposure to oxygen species (free radicals), toxins, heavy metals, microbes, UV light and other stressors [5]. Thus, HSP70 is continuously present in the human serum in daily life. In addition, HSP70 is a molecular chaperone which normally promotes the self-assembly of newly synthesized polypeptide chains of proteins into a native spatial structure, assembly of protein complexes (protein folding), and transport of proteins through membranes, as well as the participation of proteins in signal transduction [6]. Furthermore, release of endogenous HSP molecules captured by dendritic cells (DCs) from some cells can present HSP70-associated peptides on the major histocompatibility complex (MHC) by cross presentation and thereby promote cytotoxic T-lymphocytes (CTLs) and antibody production [7].

It has been reported that serum HSP70 levels are elevated in subjects with inflammation as compared to those without inflammation [8]. Therefore, activation of the *HSP* gene is closely associated with various diseases characterized by inflammation. As the expression and production of HSP70 can be strongly induced by various stressful stimuli, significant upregulation of HSP70 has been found in various diseases associated with inflammation. As a result, increased serum HSP70 levels are also known to be involved in the progression of multiple diseases associated with inflammation, including atherosclerosis, heart failure, and diabetes [9-11].

This is the first study to report a decrease of the serum HSP70 levels after "ENEX" inhalation. However, the precise mechanism(s) underlying the decrease of the serum HSP70 levels following "ENEX" inhalation remains unclear at the present time. When steam-gas mixture containing H_2 gas, or "ENEX," is administered by inhalation via a nasal cannula to the subjects, it could be expected to rapidly diffuse into the body and exert antioxidant actions (scavenging of free radicals), which could underlie the significant reduction of the serum HSP70 levels. Our findings strongly suggest that "ENEX" could be potentially beneficial for preventing/treating various diseases caused by acute or chronic inflammation and come to be used widely in clinical settings in the future. In addition, the kinetic changes of the serum HSP70 are thought as an excellent indicator in various disease conditions associated with inflammation.

In conclusion, we are the first to report that inhalation of a steam-gas mixture containing H_2 gas, known as "ENEX," decreased the serum concentrations of HSP70, an inflammatory biomarker. Further analyses, at both the basic and clinical levels are needed to clarify in detail, the mechanism(s) underlying the decrease of the serum HSP70 levels following "ENEX" inhalation.

Acknowledgements

We wish to thank the medical research team members (Hashimoto, K and Hidaka, N) of Suisonia Co. for their excellent technical support.

Disclosure

None of the authors has any conflict of interest to disclose.

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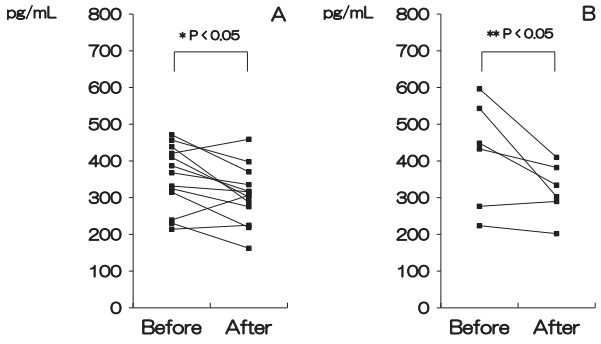

Figure 1. Measurement of serum HSP70 in the male or female volunteers
Measurement of HSP70 in serum samples obtained from the male volunteers (n = 13) (panel A) or the female volunteers (n = 6) (panel B) was performed using a HSP70 detection EIA kit. The measurements were performed in triplicate. *P < 0.05 (before inhalation vs. after inhalation). **P < 0.05 (before inhalation vs. after inhalation).

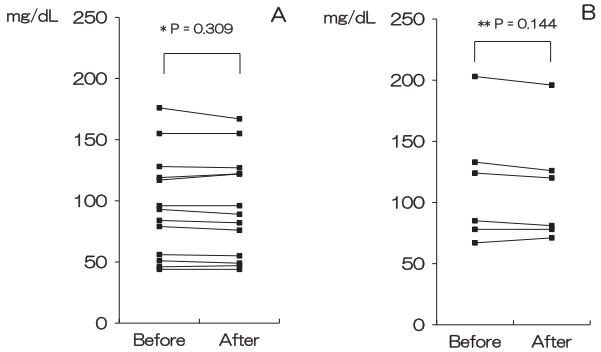

Figure 2. Measurement of serum total IgM in the male or female volunteers
Measurement of the serum total IgM in serum samples obtained from the male volunteers (n = 13) (panel A) or the female volunteers (panel B) was outsourced to SRL. The measurements were performed in triplicate. *P = 0.309 (before inhalation vs. after inhalation). **P = 0.144 (before inhalation vs. after inhalation).

水素ガスを含む蒸気-混合ガス吸入後の血清中heat shock protein 70 (HSP70)の動態解析

池脇 信直　園田 徹 *　山尾 裕道 **

九州保健福祉大学生命医科学部生命医科学科・順正学園免疫学研究所　〒882-8508　宮崎県延岡市吉野町1714-1
*九州保健福祉大学保健科学部作業療法学科・順正学園免疫学研究所　〒882-8508　宮崎県延岡市吉野町1714-1
**井上病院　〒887-0866　宮崎県延岡市平原町1-990-1

要旨

水素ガス（濃度 0.1～ 0.3%）を含む蒸気 -混合ガス“ENEX” を鼻カニューラで吸入した。吸入後、血清中の炎症性バイオマーカーである heat shock protein 70 (HSP70)の動態を酵素抗体法（enzyme immunoassay：EIA）で解析した。その結果、“ENEX” 吸入 60分後、血清中の HSP70の濃度が男女共に吸入前と比較して統計学的に有意な減少が認められた（$P<0.05$）。一方、血清中の総 IgM量は吸入前後で男女において統計学的な有意差は認められなかった（男：$P=0.309$、女：$P=0.144$）。以上の結果は、“ENEX” が鼻粘膜を経由して、生体に発生する活性酸素を除去すると共に、炎症反応を抑制させる作用があることを示唆するものである。将来的には炎症反応によって発症する様々な疾病の予防や治療に応用できる可能性があると考えられる。

キーワード：ENEX、ヒートショックプロテイン 70、水素ガス、炎症、総 IgM